OSMOTIC BEHAVIOR IN AN INTERTIDAL LIMPET, ACMAEA LIMATULA[1]

EARL SEGAL AND PAUL A. DEHNEL

Department of Biology, Rice University, Houston 1, Texas, and Department of Zoology, University of British Columbia, Vancouver 8, B. C.

Marine invertebrates generally are considered to be poikilosmotic. Varying degrees of ion and/or volume regulation have been demonstrated in decapod Crustacea, polychaetes, and a few other selected groups. Little information is available on the osmotic behavior of molluscs. Bethe (1934) has shown that in 75% sea water the nudibranch *Doris* possesses essentially no volume regulation and loses salts, in the form of chloride, over a 48-hour period. In the tectibranch, *Aplysia,* Bethe (1929, 1930, 1934) concluded that the response to 75% sea water is an initial weight increase, and a subsequent loss in weight as salts and water are lost from the body. But van Weel (1957) reported that *Aplysia* could tolerate only 95% sea water and that further dilutions seriously damaged the animals. *Onchidium,* a marine pulmonate, swelled in dilute sea water but regained its original weight after return to normal sea water, demonstrating that little salt was lost (Dakin and Edmonds, 1931). No data appear to be available for prosobranch gastropods.

In intertidal prosobranch gastropods, volume changes in response to increased salinities seemingly cannot be separated from volume changes due to desiccation brought about by exposure. This problem is particularly acute in species with a relatively wide intertidal vertical distribution. All prosobranchs possess an extra-visceral space between the shell and soft parts. Water retained in this space serves as a jacket around the head, ctenidia, portions of the visceral mass and foot, and may serve to retard desiccation. Also, in a study of water relations in the limpet, *Acmaea limatula* Carpenter, Segal (1956) suggested that the extra-visceral space may have an osmoregulatory function.

The object of this study is to determine whether the limpet *Acmaea limatula* is capable of osmotic regulation over a range of salinities, and to determine whether extra-visceral water serves (1) an osmoregulatory function, *i.e.,* maintains an ion and/or water gradient between the blood and the external medium, and (2) as a temperature buffer.

Material and Methods

Acmaea limatula used in this study were collected from the following three geographic locations: Dike Rock, immediately north of Scripps Institution of

[1] This study was aided by grants from the National Science Foundation, Grant Nos. G-5943 and G-9348. We gratefully acknowledge the equipment and space provided by Drs. Denis L. Fox, Carl L. Hubbs and Per F. Scholander, Scripps Institution of Oceanography, University of California, La Jolla, California. We wish to thank Dr. Warren J. Gross and Dr. C. L. Prosser for their critical reading of the manuscript.

Oceanography, La Jolla, California (32°, 52′ N Lat.; 117°, 15′ W Long.); northeast side of Punta Banda (Todos Santos Bay), Baja California (31°, 44′ N Lat.; 116°, 42′ W. Long.); southwest side of Punta Banda (Papalote), Baja California (31°, 44′ N. Lat.; 116°, 43′ W Long.). Dike Rock is on the open coast, with a mean inshore summer temperature range from 19° to 21° C., mean winter temperature, 15° to 17° C. The northeast side of Punta Banda is a protected warm water coastal bay with a summer inshore temperature range from 21° to 25° C. The southwest side of Punta Banda is a cold-water, upwelling open coast area with a summer inshore temperature range from 14° to 16° C. During the winter months the mean difference in inshore water temperatures between the two sides of Punta Banda is less than during the summer months (Todos Santos Bay is only 1° to 3° C. warmer). Mean temperature values for summer and winter for Punta Banda were obtained from Hubbs (personal communication).

Salinity experiments

Animals collected at Dike Rock were returned to the laboratory and placed directly into the experimental salinities, 25, 50, 75, 125 and 150% sea water, at a cold room temperature of 20° C. All animals were kept in the dark, not fed and maintained in plastic containers (about 3.5 liters sea water each), approximately 30 animals per container. Animals collected from Punta Banda were placed into salinities of 50 and 150% sea water. Required temperature (20° C.) was maintained in a dry ice box.

Salinities above 100% sea water were obtained by freezing normal sea water. The concentrated sea water was then diluted to the required salinities with distilled water. Salinities below 100% sea water were obtained by adding distilled water to normal sea water. All field and experimental salinities are expressed as percentage sea water, based on a standard sea water, 33.70‰ salinity, 18.65‰ chlorinity at 20° C. Salinities were determined on a 1000-cycle conductivity bridge calibrated to the standard sea water noted above.

Blood was sampled by making an incision through the ventral surface of the foot into the ventral sinus. Blood was collected in 0.4-mm. (I.D.) tubes, sealed with Nevastane grease and quick frozen on dry ice. Mucus, residual water and debris were removed carefully from the foot before the incision was made. Blood of animals from Dike Rock was sampled at three, 24 and 48 hours after immersion at the various salinities. Blood of animals from Punta Banda was sampled three hours after immersion. Blood from control animals (100% sea water) for both Dike Rock and Punta Banda experiments was obtained immediately upon removal of the animals from the normal habitats. The value determined for the control animals served as a baseline, and this was essentially 100% sea water. A modified method for melting point determination, as described by Gross (1954), was used to determine total osmotic pressure.

Field desiccation experiments

At Dike Rock, experiments were conducted on July 13 and July 15, 1960. On July 13 approximately 150 animals were collected, randomly divided for individuals and size and placed on grey lava and grey sandstone above the high tide

level for that day. All limpets attached immediately. Blood was collected in the same manner as described for the salinity experiments. Extra-visceral water is the volume of water which is contained within the mantle cavity and surrounds the visceral mass and the foot. At the time of collecting animals this extra-visceral water is essentially sea water, and is in equilibrium with body fluids. Extra-visceral water was collected by thoroughly damp-drying the limpet, then gently applying pressure to the foot. Both blood and extra-visceral water samples were collected at the same time and handled in the same manner. Blood and extra-visceral water samples from the experimental animals were collected at two-hour intervals over a six-hour period. All samples were frozen immediately.

On July 15 approximately 150 animals were collected, half of which were placed on grey sandstone. The remaining 75 animals were shaken and blotted to remove as much of the extra-visceral water as was feasible. Then, they were placed near the first group. Blood and extra-visceral water samples from the experimental animals were collected at two-hour intervals over a six-hour period.

On July 27 approximately 150 animals were collected from Papalote (Open Coast), Punta Banda and returned to Todos Santos Bay. These animals were marked and placed at the level occupied by individuals of the Bay population. On July 28 approximately 100 animals each of the Todos Santos Bay and Papalote populations were removed from the bay and placed on light sandstone above the high water mark for that day. Blood and extra-visceral water samples from the experimental animals were collected at two-hour intervals over a six-hour period. Samples were frozen and returned to Scripps for analysis.

For all field experiments blood and extra-visceral water samples from control animals were taken at the time the experimental animals were removed from the intertidal region. These samples served as the baseline values, namely 100% sea water.

Rock surface and animal temperatures were obtained with a thermistor probe.

Weight change experiments

To determine the change in water content, expressed as per cent body weight, twenty animals each from Dike Rock were placed in 50, 100 and 150% sea water in the dark, at 20° C., for a period of 24 hours. Total damp-dried wet-weight was determined before and after animals were placed at the different salinities. After 24 hours, soft parts were removed from the shell and both soft parts and shell were weighed separately. Shell and soft parts were dried to constant weight at 110° C. for 24 hours and dry-weight of each was determined.

All animals used for the salinity, field desiccation and weight-change experiments were collected from ecologically similar intertidal locations; they were submerged at approximately a 1.5-foot tide.

Results

Salinity experiments

The data shown in Figure 1 for Dike Rock animals demonstrate conclusively that *Acmaea limatula* does not regulate osmotically to either low or high salinities. Isotonicity is reached within 24 hours over the range of salinities. After this

24-hour period the concentration difference between blood and external medium was approximately 5% sea water, a gradient to which no significance is attached. At all of the salinities, except 25% sea water, no natural deaths occurred within 48 hours. Within six hours limpets held at 25% sea water were extremely swollen. These animals were all dead by 24 hours. Body fluid concentrations

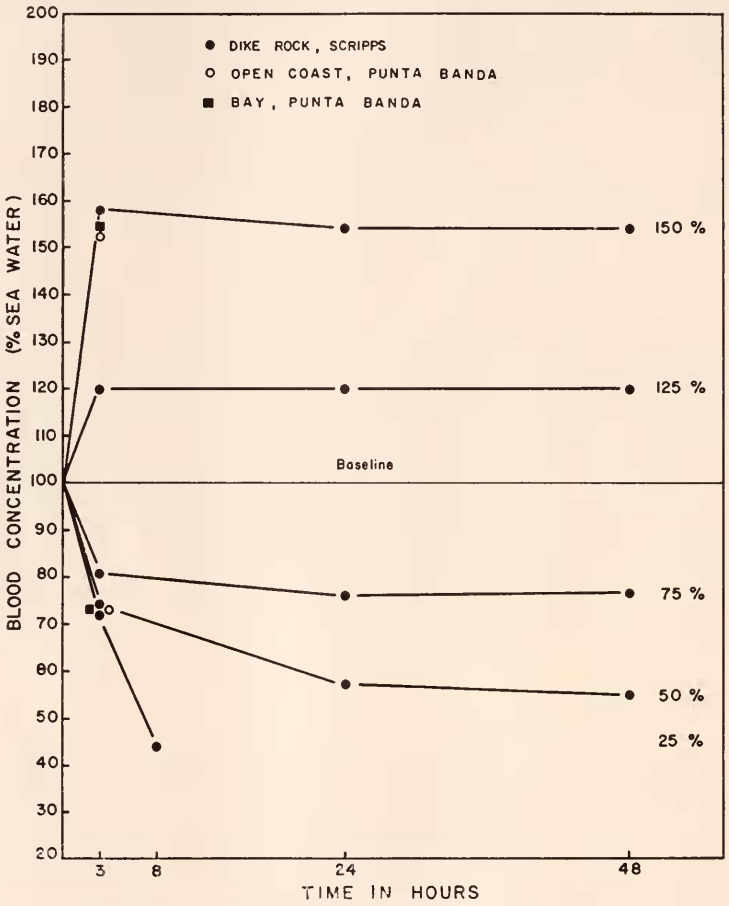

FIGURE 1. Change in blood concentration, expressed as per cent sea water, as a function of time, in hours, for three populations of *Acmaea limatula*. Each point is the mean value for at least ten animals. Line at the 100% sea water value represents the baseline for the three populations. Experiments were run at 20° C.

of control animals for all salinity experiments at the three collecting areas were determined, and differed only slightly from 100% sea water. No biological significance is attached to these differences. Animals in 125 and 150% sea water were isotonic with the media in three hours; in 50 and 75% sea water they were not. Furthermore, the lower the external concentration, the greater the gradient between blood and external medium. For example, at three hours, animals in

75% sea water have approached 8% sea water of being isotonic with the medium; in 50% sea water, 48%; in 25% sea water, 70%.

Examination of Figure 1 shows a slight hypertonicity at 150% and 50% sea water, and hypotonicity at 125% sea water after 24 hours. It could be suggested that this demonstrates a weak hyper-osmotic regulation. However, these points represent an average, and the variability of individual blood concentrations would seem not to permit this conclusion, based on these data alone. Further, at the two higher salinities, blood concentration is absolutely higher in one and lower in the other, showing no constancy toward hyperosmotic regulation.

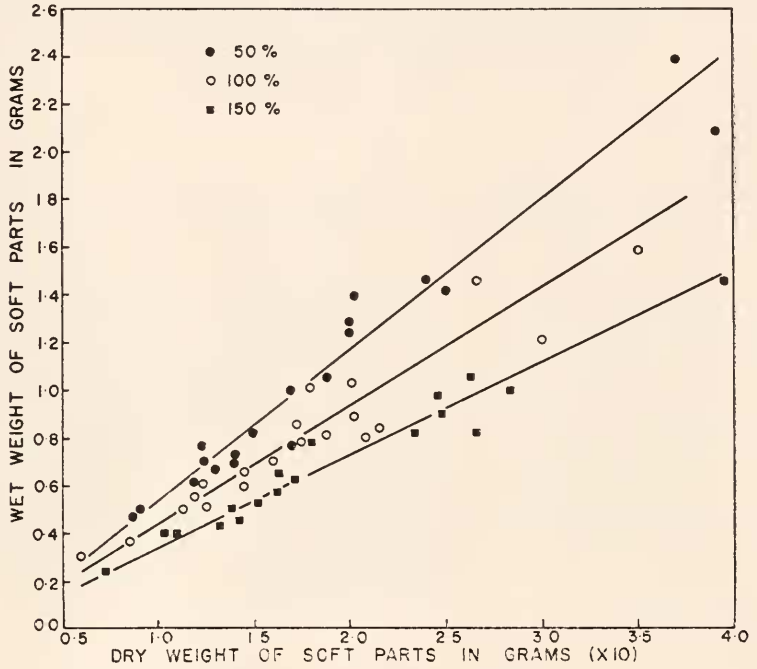

FIGURE 2. Relation between the wet-weight of soft parts and the dry-weight of soft parts, in grams, for *A. limatula* from Dike Rock, Scripps Institution. The points represent individuals maintained at 50, 100, and 150% sea water for 24 hours and *b* values are 0.63, 0.50, and 0.40, respectively.

Because of the absence of osmoregulation in animals from Dike Rock, Punta Banda animals were tested at two salinities (50 and 150% sea water) for no longer than a three-hour period. The osmotic responses of limpets from the two sides of Punta Banda and Dike Rock essentially are identical (Fig. 1).

Weight change experiments

Figure 2 is a plot of the wet-weight of soft parts as a function of dry-weight of soft parts after a 24-hour period at 50, 100 and 150% sea water. Analysis of these data has shown that the wet weight-dry weight relationship is best described by a straight line. Slope values (b), as determined by the method of least

squares, for these lines are 0.63, 0.50, and 0.40 for 50%, 100%, and 150%, respectively. Comparison of any combination of two salinity regression lines has shown differences to be statistically significant ($P = 0.01$). Linearity of the curves shows that at each salinity there is a constant ratio of wet-weight to dry-weight over the weight range shown. Therefore, percentage body water shows

TABLE I

Changes in wet weight, dry weight and total water as per cent body weight in Acmaea limatula in 50%, 100% and 150% sea water. Total wet weight values were obtained before immersion in the experimental salinities, 50% and 150% sea water. Wet weight of soft parts (1) resulted when shell weight was subtracted from total wet weight. Wet weight of soft parts (2) were values obtained after 24 hours immersion in the experimental salinities.

50% SW					100% SW				150% SW				
Total wet weight (gms.)	(1) Wet weight soft parts (gms.)	(2) Wet weight soft parts (gms.)	Dry weight soft parts (gms.)	Total water % body weight	Total wet weight (gms.)	Wet weight soft parts (gms.)	Dry weight soft parts (gms.)	Total water % body weight	Total wet weight (gms.)	(1) Wet weight soft parts (gms.)	(2) Wet weight soft parts (gms.)	Dry weight soft parts (gms.)	Total water % body weight
1.4207	0.6472	0.7133	0.1352	81.04	3.0436	1.2236	0.3018	75.34	2.9028	1.3186	0.9898	0.2828	71.42
1.5450	0.7315	0.7739	0.1725	77.71	1.2995	0.6082	0.1453	76.10	4.4532	1.7805	1.4586	0.3952	79.90
1.3980	0.5790	0.6618	0.1283	80.61	1.9273	0.7782	0.1748	77.53	1.3180	0.6374	0.4705	0.1415	69.92
1.4146	0.6696	0.8147	0.1530	81.22	0.6928	0.3196	0.0613	80.81	1.0209	0.4618	0.3851	0.1070	72.21
1.3037	0.6037	0.6068	0.1171	80.70	1.5436	0.7073	0.1598	77.40	2.7523	1.2181	0.9756	0.2476	74.62
2.7468	1.1931	1.4266	0.2508	82.41	0.8514	0.3749	0.0846	77.43	2.2798	0.8765	0.7872	0.1779	77.40
2.3510	1.0437	1.2655	0.2017	84.06	2.0868	1.0176	0.1794	82.37	1.5970	0.7123	0.6284	0.1709	72.80
0.8396	0.4208	0.4790	0.0865	81.94	1.8631	0.8167	0.1877	77.01	2.6243	1.1586	0.8904	0.2476	72.19
2.3088	0.8665	1.0714	0.1707	84.06	1.9100	0.9144	0.2030	77.79	3.7538	1.5395	1.4618	0.3352	77.06
1.3292	0.6040	0.7077	0.1392	80.33	1.1983	0.5629	0.1208	78.53	2.1562	1.0776	0.8202	0.2338	71.49
0.9147	0.4074	0.4982	0.0895	81.96	1.6588	0.8425	0.2164	74.31	2.9687	1.2395	1.0636	0.2616	75.98
1.2538	0.5692	0.7081	0.1238	82.51	2.1856	1.0391	0.2018	80.57	2.1477	0.8917	0.8215	0.2657	67.65
2.0858	1.0010	1.2969	0.2034	84.31	1.0259	0.4963	0.1254	74.73	1.6088	0.7073	0.6566	0.1625	72.25
4.4188	1.7264	2.3998	0.3680	84.66	2.0205	0.8192	0.2079	74.62	1.0597	0.4649	0.3922	0.1025	73.86
1.0942	0.5563	0.7553	0.1230	83.71	3.5489	1.4679	0.2655	81.91	1.2498	0.5712	0.5108	0.1390	72.78
2.0348	0.8913	1.0694	0.1880	82.42	2.5193	0.8456	0.1728	79.56	1.6555	0.6627	0.5277	0.1511	71.36
2.4626	1.0270	1.4187	0.2071	85.40	1.2226	0.6255	0.1186	81.03	1.6388	0.6815	0.5650	0.1615	71.41
2.4067	1.2127	1.4654	0.2386	83.71	3.4640	1.5934	0.3513	77.95	0.6682	0.2968	0.2405	0.0731	69.60
					1.0167	0.4857	0.1144	76.44	1.1357	0.5198	0.4413	0.1307	70.38
					1.3939	0.6740	0.1454	78.42					
Mean —	—	—	—	82.38	—	—	—	77.99	—	—	—	—	72.86
S.D. —	—	—	—	1.92	—	—	—	2.49	—	—	—	—	3.02
σ_m —	—	—	—	0.45	—	—	—	0.56	—	—	—	—	0.69

	50%–100% S.W.	50%–150% S.W.	100%–150% S.W.
df	36	35	37
t	6.115	11.513	5.773
P	0.001	0.001	0.001

no weight dependence at any of the salinities. The mean body water (expressed as percentage of body weight) has been determined, and is 78% for baseline animals from 100% sea water, 82% for animals from 50% sea water and 73% for animals from 150% sea water (Table I). Therefore, there was an average 28% *increase* in body water in 50% sea water and approximately the same *decrease* (28%) in 150% sea water when both are compared with the 100% sea water baseline. These calculations are based on the fact that for a given weight animal,

for example 100 grams, in 100% sea water, 78 grams are water and 22 grams are non-water. This latter value is essentially a constant. In 50% sea water, 82% of the animal is water and 18% is non-water, which in turn is 22 grams. Then, if 22 grams equals 18%, 82% equals 100 grams of water, or a gain of

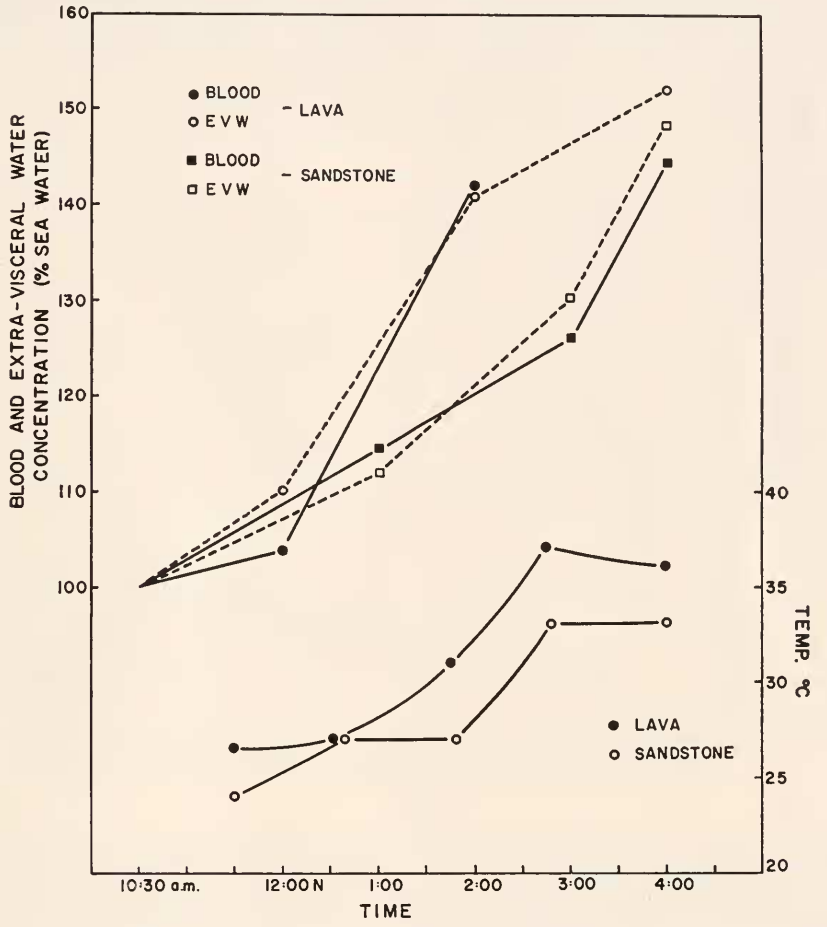

FIGURE 3. Change in concentration of blood and extra-visceral water of *A. limatula* placed on grey lava and grey sandstone out of water. Each point is the mean value for at least ten animals. Blood concentration at beginning of experiment was 99.4% sea water. Experiment was performed on July 13, 1960, at Dike Rock, Scripps Institution.

22 grams, which is the 28% increase in the body water of the animal. The same argument must be considered for the animal in 150% sea water. In this instance there is a decrease in the total body water, but the non-water component remains constant. Therefore, the per cent increase and decrease in body water can be the same, although absolute mean body water can differ in the experimental salinities.

When the mean body water, expressed as per cent body weight of the three groups of animals in different salinities, is compared in all combinations, the differences are highly significant ($P = 0.001$, see Table I).

Weights of animals shown in Figure 2 and Table I are considered to be representative of the populations of this limpet, and the changes shown must be assumed to be representative of the response any individual would demonstrate if successive wet and/or dry weights of soft parts in two different salinities could be obtained.

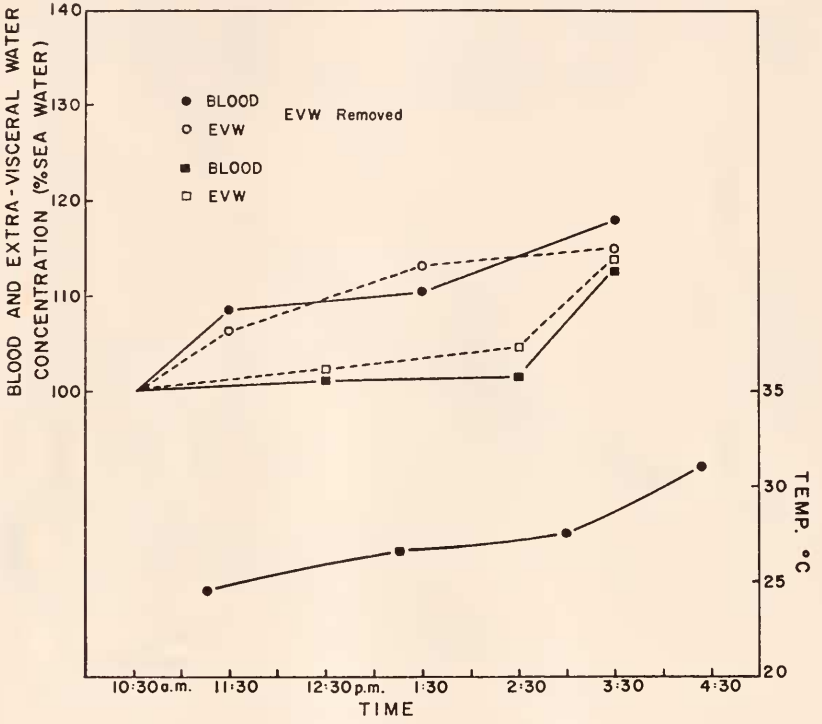

FIGURE 4. Change in concentration of blood and extra-visceral water of *A. limatula* when extra-visceral water was removed, as compared with *A. limatula* from which the extra-visceral water was not removed. Both groups were on grey sandstone out of water. Each point is the mean value for at least ten animals. Blood concentration at beginning of experiment was 99.6% sea water. Experiment was performed on July 15, 1960, at Dike Rock, Scripps Institution.

Total wet weight in Table I was measured before animals were immersed in the experimental salinities. Wet weights of soft parts (1) were body weights before immersion, and were determined by subtracting shell weight from total wet weight. Shell weight does not change and dry weight of shell differed from wet weight by less than 1%, and this difference was statistically insignificant. Shell weight (based on approximately sixty animals) represented 55.23% of total weight. Wet weight of soft parts (2) was determined after 24 hours immersion in the experimental salinities, 50% and 150% sea water.

Field desiccation experiments

Dike Rock

Figure 3 shows the effect of exposure of animals on two different substrates. It becomes apparent immediately that the rate of change of the concentration of the blood and extra-visceral water of animals on lava is much greater that that of animals on sandstone of the same color. The temperature record indicates that this more rapid rate of change on lava is a direct effect of the more rapid rise in temperature and higher values reached on the lava as compared with the sandstone. The rise in blood concentration parallels the rise in extra-visceral water for animals measured on sandstone. The same parallelism occurs with animals on lava. Based on the anatomy of the limpet, the extra-visceral water is in immediate contact with the environment and it would be expected, *a priori*, that a change in the extra-visceral water would occur first. In general this is what occurs in all three field experiments. At the last blood sampling from animals on the lava we were unable to obtain blood. However, extra-visceral water was still present. This suggests that with extreme desiccation there may be a movement of fluid from the sinuses within the animal to the extra-visceral space.

The remaining experiments were conducted on sandstone because this substrate is the more typical one both at Dike Rock and Punta Banda. Further, the rate of rise of temperature on the sandstone is slower than on lava. Figure 4 compares the response of a group of animals which have been shaken and damp-dried with that of a group of animals which have been placed directly on the substrate. The concentration of both extra-visceral water and blood was higher in the group which had a portion of the extra-visceral water removed. However, at the end of the experiment there was no difference in the concentrations of the extra-visceral water and blood of the two groups. The sudden increase in blood and extra-visceral water concentrations between 2:30 P.M. and 3:30 P.M. for the groups from which extra-visceral water was not removed was due probably to the rather abrupt temperature rise. Because of the differences in temperatures recorded for the two days at Dike Rock it is impossible to compare the results obtained from animals on sandstone for these two days.

Punta Banda

Comparison of the two populations of animals from opposite sides of Punta Banda under identical conditions of exposure showed that the blood and extra-visceral water concentrations of the Bay (Todos Santos) population increased more rapidly, remained at higher concentrations (a difference ($P = 0.01$) of 10% sea water in each case), than that of the Open Coast (Papalote) population (Fig. 5). This difference was evident throughout the period of exposure. It was noted that at the end of the experiment the two groups appeared very different. The Open Coast group had more fluid in the mantle tissues, and it was more difficult to obtain blood samples from the Bay group.

Discussion

The data show that the intertidal gastropod *Acmaea limatula* does not osmoregulate over the range of salinities, from 25% to 150% sea water (Fig. 1). The

relative blood concentration and weight changes shown for *A. limatula* in hypotonic media are similar to that reported for *Doris* (Bethe, 1934) and for *Onchiduim* (Dakin and Edmonds, 1931). However, *A. limatula* differs during the course of our experiments (48 hours) from that reported for *Aplysia* (Bethe, 1930). *Aplysia* shows an initial weight change in low and high salinities, but within a few hours approaches its original weight due to a subsequent gain or loss of ions. *Acmaea limatula* at 24 hours in 50% sea water shows a weight gain and in 150%

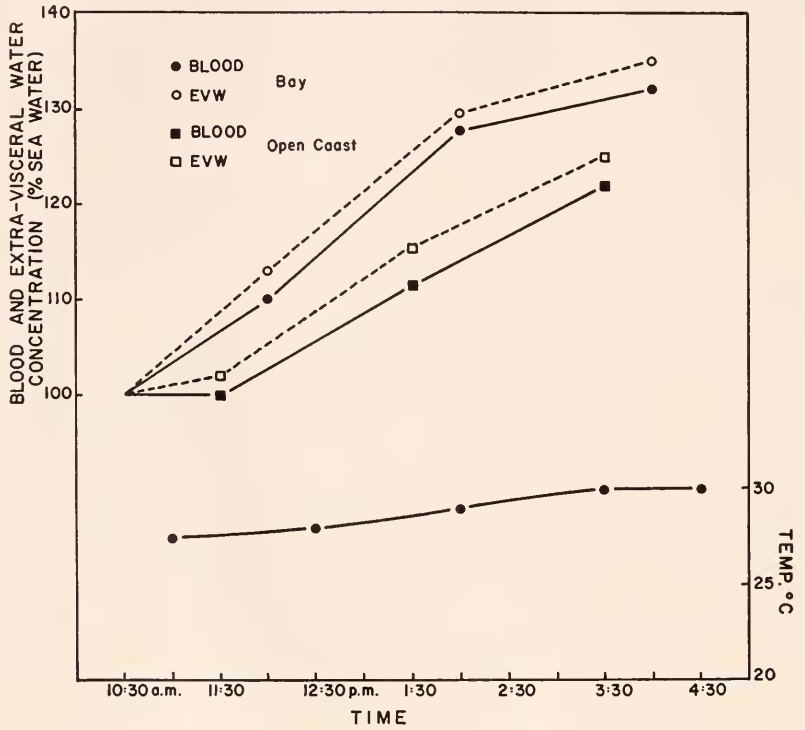

FIGURE 5. Change in concentration of blood and extra-visceral water of *A. limatula* from the Bay and Open Coast sides of Punta Banda, Baja California. Open Coast animals spent 24 hours in the Bay intertidal zone before experiment. Both groups were on light-colored sandstone. Each point is the mean value for at least ten animals. Blood concentration of both groups at beginning of experiment was 99.5% sea water. Experiment was performed July 28, 1960, at Todos Santos Bay, Punta Banda.

sea water shows a weight loss (Fig. 2). In the same time period and salinities there is a corresponding increase and decrease in blood concentration which remains isotonic with the medium for an additional 24 hours. Although weight changes were not followed beyond 24 hours, it is probable that the body weight would not have returned to normal. However, Gross (1954) has shown that the sipunculid *Dendrostomum* remains isotonic in various sea water dilutions but can return to normal weight.

It has been demonstrated (van Weel, 1957) that *Aplysia juliana* can regulate weakly in 95% sea water. He criticized the results on *Aplysia* reported by Bethe

(1930) and has suggested that the low salinity (75%) to which the animals were subjected was a non-physiological condition. Further, van Weel contended that since weights of some animals, after osmotic equilibrium was established in 75% sea water, were less than initial weights, this condition reflected active water removal, and hence weak regulation. Van Weel also has re-evaluated results for *Doris* (Bethe, 1934) and interpreted failure to reach osmotic equilibrium in 75% sea water, as measured by blood chloride concentration, to mean that the blood remained hypertonic, and the animals showed some regulation.

Gross (1954) in his work on *Dendrostomum* has shown that this sipunculid responds superficially as an osmometer, and has discussed this isotonic relation of blood to various sea water concentrations, based presumably only on water fluxes, in a hypothetical manner, determining mobile (osmotically active) water by the following:

$$V = \frac{C_2 \quad (\Delta W)}{C_1 \; - \; C_2}$$

V is the volume of mobile water in per cent body weight (soft parts), C_1 is the concentration of body fluids in, for example, an external salinity of 100% sea water, and C_2 is body fluid concentration after exposure to a different external salinity, both expressed as per cent sea water, and ΔW is the change in weight after a steady-state is attained due to change in salinity. This relationship suggests whether blood concentration changes result from water or water and ion fluxes, when an external concentration change is effected.

Assume a volume of solution which is essentially constant among animals in 100% sea water, into which only water moves or leaves when the salinity of the external medium is changed. If the concentration change (C_1-C_2) of body fluids and the volume change (ΔW) of animals are known, one can calculate the original volume of the solution for the animal in 100% sea water. If only water were effecting the change, then the value for V would be the same whether the animal were in 50% or 150% sea water. However, if salts also were leaving or entering, values for animals in these two external salinities would differ.

In order to calculate mobile water values for each individual it is necessary to determine change in weight (ΔW) expressed as per cent body weight from one sea water concentration to another. Reference to the wet weights of soft parts (1) before immersion and (2) after immersion for either 50% or 150% sea water provides these data (Table I). C_2 may be obtained from Figure 1, blood concentration at 48 hours for 50% or 150% sea water. Determination of mobile water (V) follows from the above formula.

When this line of reasoning is applied to limpets in external salinities of 50% and 150% sea water, average mobile water values differ. For 50% sea water, mobile water, expressed as per cent original body weight (soft parts), equals 27.8%; for 150% sea water, 43.7%. The differences in mobile water values at these two salinities are statistically significant ($P = 0.01$). This would be interpreted to mean that salts play a greater role (at least percentage) in effecting an osmotic pressure change in the blood in 50% sea water than in 150%. In 50% sea water, salts leave and water enters the blood. If no salts were exchanged, mobile water values for both salinities should be the same, and the limpets would be responding to external salinity changes as an osmometer. In the absence of

other data, however, one cannot state definitely for animals in 150% sea water that salts are entering, as well as water leaving. Comparison of osmotically active water for a sipunculid (Gross, 1954) in high salinity water with that for the limpet in similar water shows the mobile water value for the limpet to be considerably lower. This would suggest that salts are entering the limpet in 150% sea water, thus reducing ΔW for a given concentration change. Data for the sipunculid show mobile water values for high and low salinity media to be the same.

Percentage change of body weight as water in *A. limatula* is approximately the same, 28%, in hypo- and hypertonic media. These values generally are of the same magnitude as those reported in the literature. Bethe (1930) showed that the weight of *Aplysia* increased approximately 50% in two to four hours in 50% sea water, and 20% in 75% sea water over the same time period. Dakin and Edmonds (1931) showed for *Onchidium* an 8% increase in weight in one and one-half hours at 50% sea water, and a 70% increase in 22 hours in 10% sea water.

Under conditions of air desiccation the response is very similar to that recorded for salinity dehydration. This can be seen by comparing Figure 1, three-hour, 150% sea water with Figure 3, 4:00 P.M. sample. Certainly, air desiccation is the more natural environmental stress, although in high tide pools it is likely that high salinities can be reached. Individuals of *A. limatula* living relatively high in the intertidal zone can easily withstand periods of desiccation due to tidal exposure. Body fluid concentration rises appreciably, but data from Figure 1 show that animals in 150% sea water survived for two days. We were able to collect extra-visceral water, but not blood, from limpets on lava at 4:00 P.M. The animals were shrunken, and no response to stimuli was evoked. The animals appeared to be moribund. We had no difficulty collecting blood and extra-visceral water from limpets on sandstone. The temperature on the sandstone was 5° C. lower. It seems reasonable to suggest that the higher temperature on the lava was the cause of death. Certainly, it would appear to be advantageous for *A. limatula* to frequent the sandstone as opposed to the lava, and so they do. The only conditions under which *A. limatula* were found on lava were when the lava was protected from the sun by an overhang or the limpets were in pockets of water.

The question remains whether extra-visceral water serves any osmotic function. It does appear to serve an osmotic function in that, if the animal does not have this water jacket, the body would lose water to the space more rapidly and the blood concentration would, therefore, rise more rapidly (Fig. 4). The animals in which the extra-visceral water was removed demonstrate this. At the same time, the water jacket has another function, which may well be the primary one, to serve as a temperature buffer. Since the increase in extra-visceral water and blood concentration is a direct function of the temperature (through evaporation), the differences in fluid concentration between the two groups must be due to the presence or absence of extra-visceral water.

We noted, during the later stages of desiccation, on July 13, that the limpets raised their shells off the substrate. This behavioral response was more evident among the animals on the lava (the warmer substrate). This behavior may be interpreted in two ways: an escape response or a cooling response. We are inclined to believe that it is not an escape response because we observed no animal movement approximately one-half hour after placing the animals on the rocks.

This response resembles the "stilting response" described by Alexander and Ewer (1958) in the scorpion. They concluded that the scorpion used this behavior for cooling. It may well be that this is the situation in *A. limatula*. When the animal raises its shell, the extra-visceral water surface is presented to the air. Evaporation, and, therefore, cooling, may now take place.

It is clear that the population from Todos Santos Bay and the population from the Open Coast (Papalote) did not respond similarly to identical desiccating conditions. The blood and extra-visceral water of individuals from the Bay were more concentrated throughout the experimental period. The reason for this difference is not clear. It is possible that the difference in the two populations from Punta Banda is due to a behavioral response. The Bay population is exposed generally to higher temperatures. These limpets may rise from the substrate more often for evaporation, thus losing water more rapidly. However, this behavioral response described for animals from Dike Rock was not observed at Punta Banda.

The Bay and Open Coast habitats, where these populations were collected, are different. The Bay is a warm-water body (during the time of our work the temperature ranged from 22.5° to 25° C.), with considerably reduced wave action, no macroscopic flora, reduced numbers of common intertidal species, and few individuals of those species that were present (*A. limatula*, in contrast, was abundant). All substrate was light-colored sandstone, and the salinity, during the days of our work, ranged from 85 to 90% sea water. The Open Coast habitat, on the other hand, is an area of cold water upwelling (14° C.), vigorous wave action, abundant macroscopic flora, numerous intertidal species with the individuals in great numbers, a mixed substrate of darker sandstone and lava, and salinity range from 95 to 100% sea water. The temperature differences we have recorded, although not as great during the winter months, are found throughout the year (see Material and Methods).

Pickens (personal communication) has shown an acclimation of the heart rate to temperature in populations of *Mytilus californianus* from the two sides of Punta Banda. Differences in response to short-term desiccation of the two populations of *A. limatula* may also be a reflection of the differences in temperature on the two sides. Unfortunately, there are no long-term salinity data available. We are not implying that the small differences in salinity, that we have recorded, are significant in terms of the response pattern. But we cannot discount the possible role of a difference in salinity in terms of its interaction with temperature. This has been brought out by the work of Kinne (1956) on growth and reproduction in the hydroid *Cordylophora*, Todd and Dehnel (1960) on temperature tolerance, and Dehnel (1960) on respiration in the intertidal crabs *Hemigrapsus nudus* and *H. oregonensis*. If the osmotic differences, resulting from desiccation, between the two Punta Banda populations are phenotypic, it is possible that such differences would be greater if the Open Coast group had not spent approximately 24 hours in the Bay intertidal zone. The response of the Open Coast limpets, due either to an acclimation or an environmental stress, following transfer to the Bay intertidal zone would seem only to shift in the direction of that response shown by the Bay animals, *i.e.*, the curve for the Open Coast population would approach that of the Bay group.

Summary

1. Osmotic and air desiccation experiments were conducted on three populations of *Acmaea limatula*: Dike Rock, Scripps Institution of Oceanography; Todos Santos Bay, Punta Banda, Baja California; Papalote, Punta Banda, Baja California.

2. *A. limatula* does not osmoregulate over a range of salinities from 25 to 150% sea water. Mobile water calculations show that blood concentration changes in different external salinities are effected by salt as well as by water movement, particularly at lower external salinities.

3. Body water as percentage body weight decreased 28% in 150% sea water and increased 28% in 50% sea water in 24 hours.

4. Isotonicity of the blood with the medium is reached within three hours in hypertonic media; longer periods were necessary in hypotonic media.

5. Under identical conditions of exposure, the rate of rise of blood and extra-visceral water concentration of animals on lava was faster and reached a higher concentration when compared with animals on sandstone.

6. The effect of removal of the extra-visceral water resulted in an increased concentration of both the extra-visceral water and the blood, when compared with limpets in which the extra-visceral water was not removed.

7. The two Punta Banda populations, under identical conditions of exposure, were different; the Bay population showed a more rapid increase in extra-visceral water and blood concentration than did the Open Coast population.

8. Under conditions of exposure, the extra-visceral water appears to function both in an osmotic and temperature buffering capacity.

LITERATURE CITED

Alexander, A. J., and D. W. Ewer, 1958. Temperature adaptive behaviour in the scorpion, *Opisthophthalmus latimanus* Koch. *J. Exp. Biol.*, **35**: 349–359.

Bethe, A., 1929. Ionendurchlässigkeit der Körperoberfläche von wirbellosen Tieren des Meeres als Ursache der Giftigkeit von Seewasser abnormer Zusammensetzung. *Pflüg. Arch. ges. Physiol.*, **221**: 344–362.

Bethe, A., 1930. The permeability of the surface of marine animals. *J. Gen. Physiol.*, **13**: 347–444.

Bethe, A., 1934. Die Salz- und Wasserdurchlässigkeit der Körperoberflächen verschiedener Seetiere in ihrem gegenseitigen Verhältnis. *Pflüg. Arch. ges. Physiol.*, **234**: 629–644.

Dakin, W. J., and E. Edmonds, 1931. The regulation of the salt contents of the blood of aquatic animals, and the problem of the permeability of the bounding membranes of aquatic invertebrates. *Austral. J. Exp. Biol. and Med. Sci.*, **8**: 169–187.

Dehnel, P. A., 1960. Effect of temperature and salinity on the oxygen consumption of two intertidal crabs. *Biol. Bull.*, **118**: 215–249.

Gross, W. J., 1954. Osmotic responses in the sipunculid *Dendrostomum zostericolum*. *J. Exp. Biol.*, **31**: 402–423.

Kinne, O., 1956. Über den Einfluss des Salzgehaltes und der Temperatur auf Wachstum, Form und Vermehrung bei dem Hydroidpolypen *Cordylophora caspia* (Pallas), Thecata, Clavidae. I. Mitteilung über den Einfluss des Salzgehaltes auf Wachstum und Entwicklung mariner, brackischer und limnischer Organismen. *Zool. Jahrb. Allg. Zool. u. Physiol.*, **66**: 565–638.

Segal, E., 1956. Adaptive differences in water-holding capacity in an intertidal gastropod. *Ecology*, **37**: 174–178.

Todd, M. E., and P. A. Dehnel, 1960. Effect of temperature and salinity on heat tolerance in two grapsoid crabs, *Hemigrapsus nudus* and *Hemigrapsus oregonensis*. *Biol. Bull.*, **118**: 150–172.

van Weel, P. B., 1957. Observations on the osmoregulation in *Aplysia juliana* Pease (Aplysiidae, Mollusca). *Zeitschr. vergl. Physiol.*, **39**: 492–506.